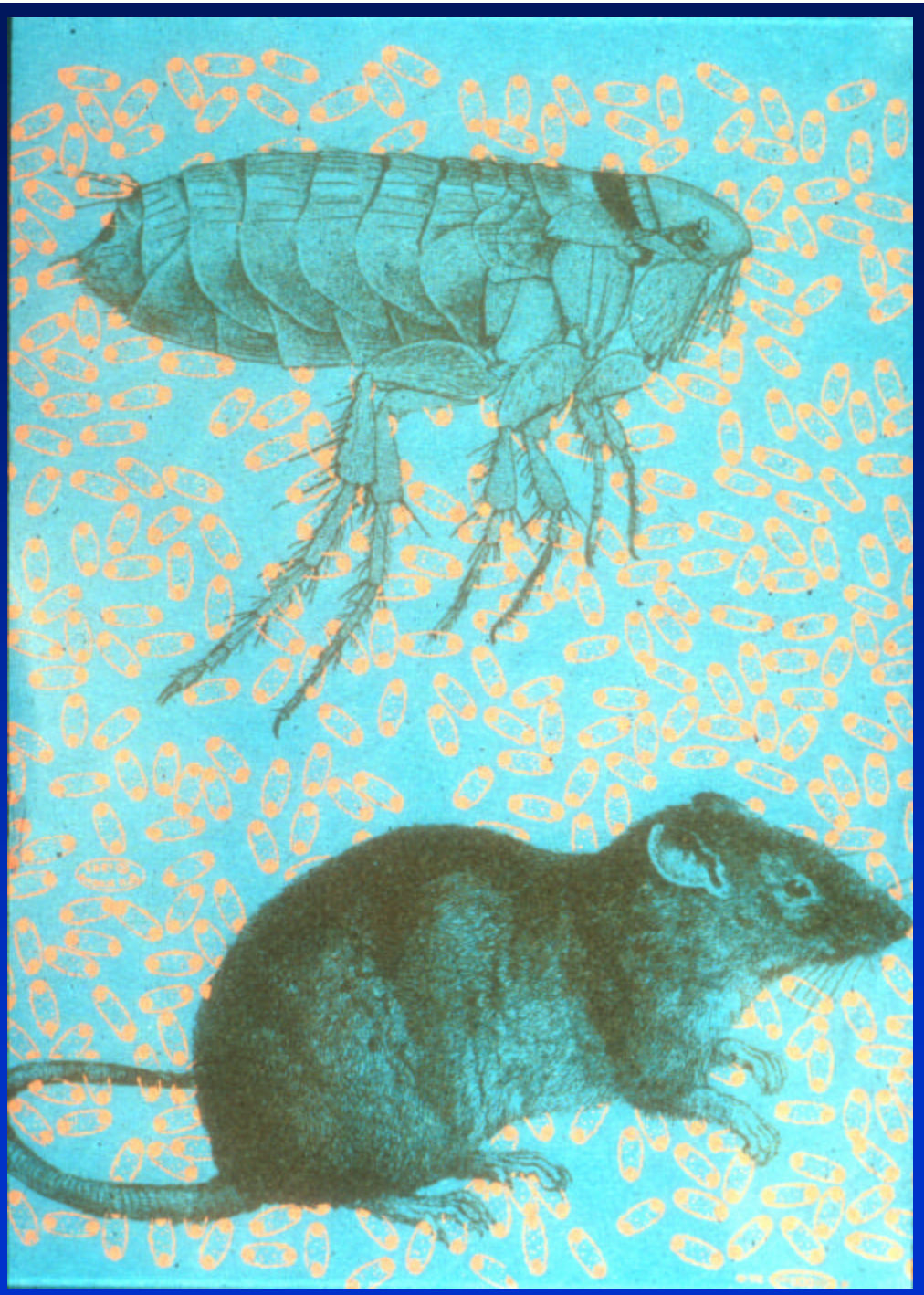
Yersinia pestis

- Cause of bubonic, pneumonic, and septicemic plague
- Gram-negative bacterium; facultative anaerobe
- Easily grown in vitro
- Genetic modifications relatively simple to engineer
- Natural foci of infection throughout the world
- Multiple-antibiotic-resistant strains have been isolated recently
- Infective by respiratory droplet route; pneumonic plague rapidly and highly fatal
- No vaccine is currently available

Bubonic Plague is a zoonotic disease with an obligate flea/ rodent/flea life cycle

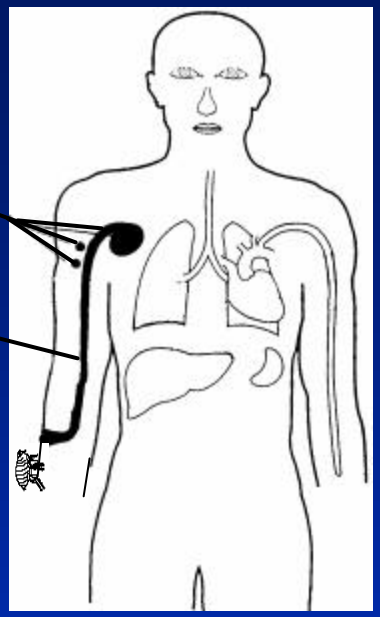


Disease Stages of Bubonic Plague

Stage 1

3. Growth in lymph node
2. Lymphatic spread

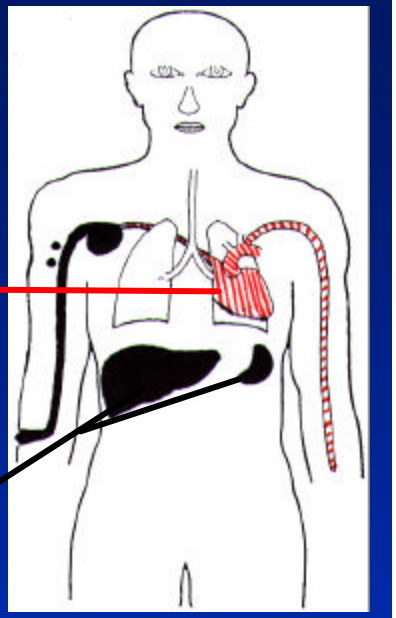
1. Entry- bite of infected flea



Stage 2

4. Hematogenous spread

5. Growth in liver and spleen

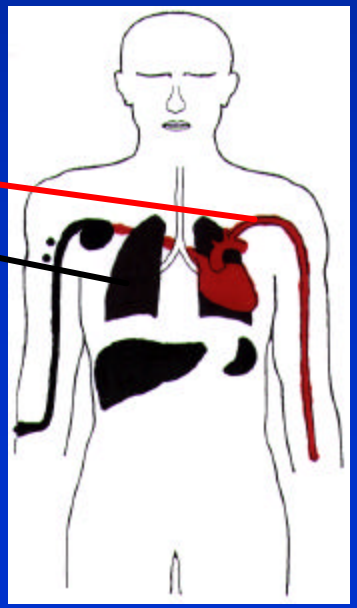


Stage 3

6. Septicemia

7. Lung infection (Occasionally)

8. Death



Symptoms

Develop 2-8 days after flea bite

- sudden onset fever, chills, weakness
- acutely swollen lymph node (bubo)
- disseminated intravascular coagulation

Bubonic Plague Virulence Factors

PROVEN VIRULENCE FACTORS

- Type Three Secretion – LvrV (immunosuppressive)
  Yop H, YopE, YopM (all antiphagocytic)
- Iron transport – Ybt and Yfe systems
- Pla protease – spread through host tissues
- PhoP/PhoQ – two component regulators
- HtrA – Heat shock serine protease

QUESTIONABLE VIRULENCE FACTORS

- F1 Capsule
- Psa fibrils

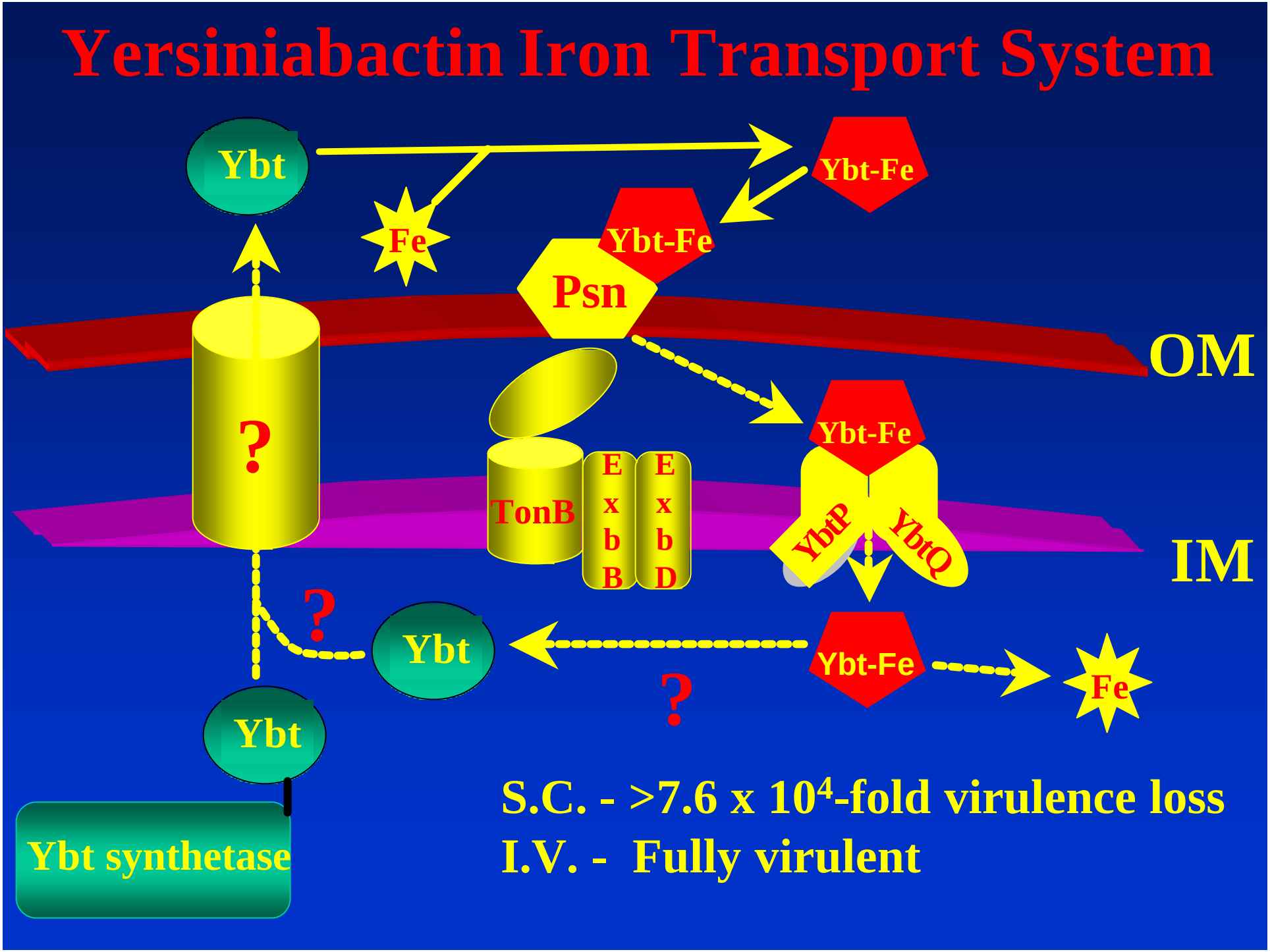
NOT TESTED

- YopT
- YpkA

NOT VIRULENCE FACTORS

- Ymt phospholipase D
- YopJ
- Hmu Heme transport
- Hms Biofilm

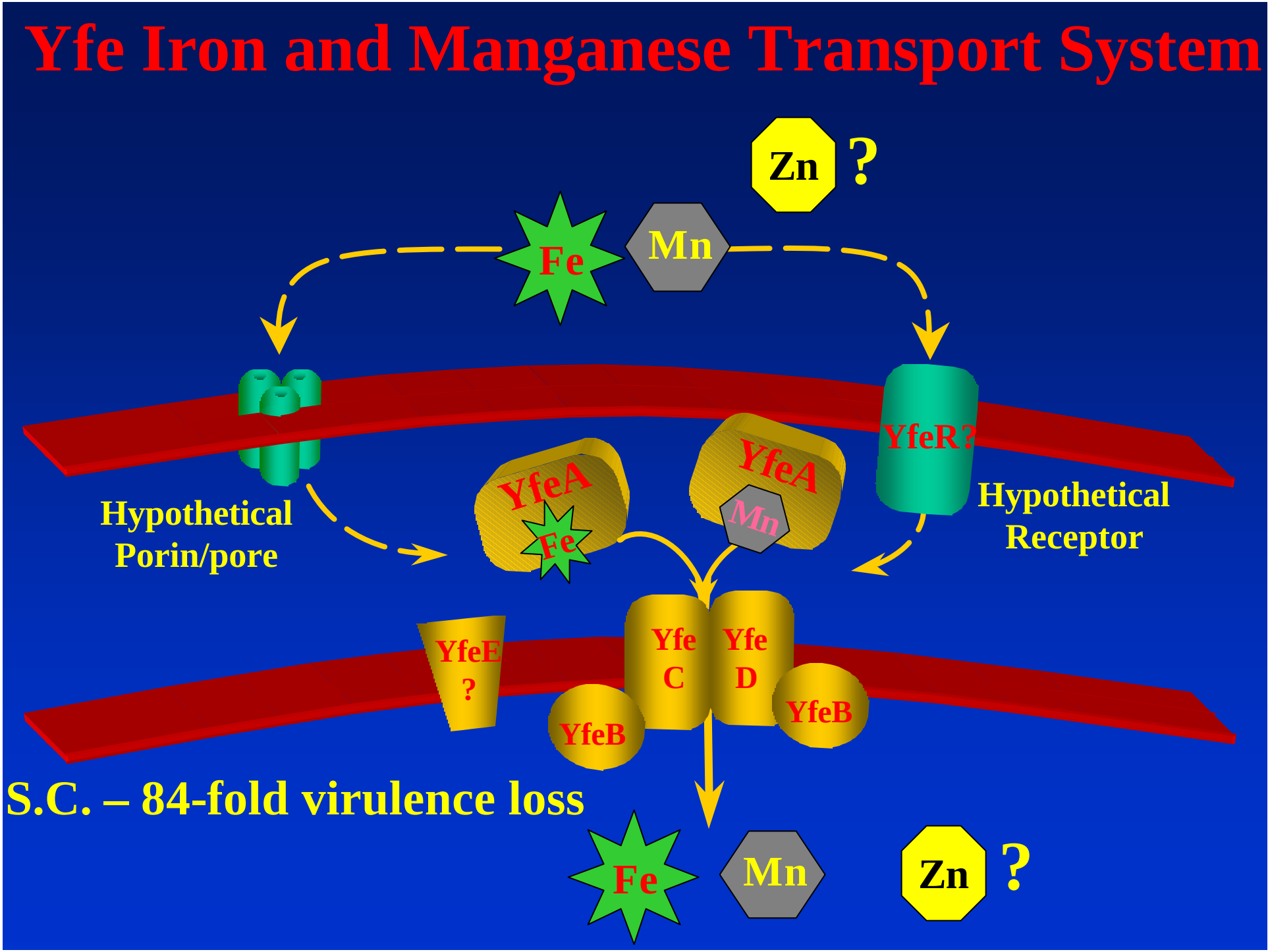
Yersiniabactin Iron Transport System



S.C. - >7.6 x 10^4-fold virulence loss

I.V. - Fully virulent

Yfe Iron and Manganese Transport System



Plasminogen activator (Pla)

- **Surface serine protease – activates plasminogen and inactivates a_2-antiplasmin**
- **Enhances adherence to extracellular matrix and laminin**
- **Enhances invasion of nonphagocytic cells**
- **Essential for virulence from peripheral routes of infecton; <u>1,400,000-fold virulence loss</u>**
- **Pla^- mutant fully virulent from IV route**

PhoP/PhoQ Two Component Regulators

- S.C. - ~75-fold virulence loss
- 2.5-fold less survival in J774 macrophage-like cells
- Significantly more sensitive to high salt conditions
- Modestly increased sensitivity to low pH and H_2O_2
- Numerous changes in protein expression
- Lipid A structure lacking aminoarabinosyl residues

HtrA Heat Shock Serine Protease

- S.C. – 12-fold virulence loss
- Numerous changes in protein expression
- Slower growth at 37ºC

F1 Capsule

- S.C. – No change in LD_{50} but ~doubled time to death
- In vitro resistance to phagocytosis
- Major immunogen and protective antigen
- Increased expression at 37ºC
- Mutants generated that secrete F1

Psa Fibrils (pH6 Antigen)

- I.V. – 214-fold virulence loss
- S.C. – little or no virulence loss
- Forms fibrillar structures at 37ºC and acidic conditions
- Expressed in macrophages
- Recombinant Psa binds human IgG

Disease Stages of Bubonic Plague

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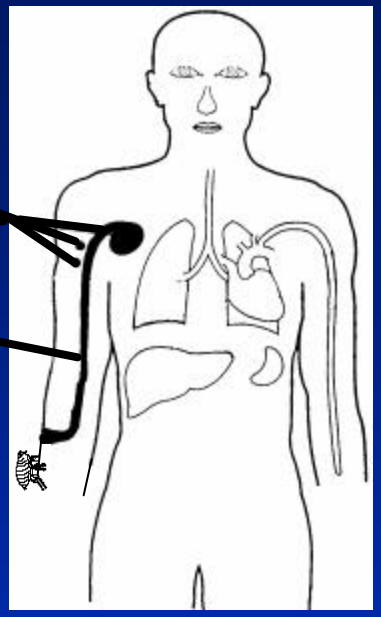
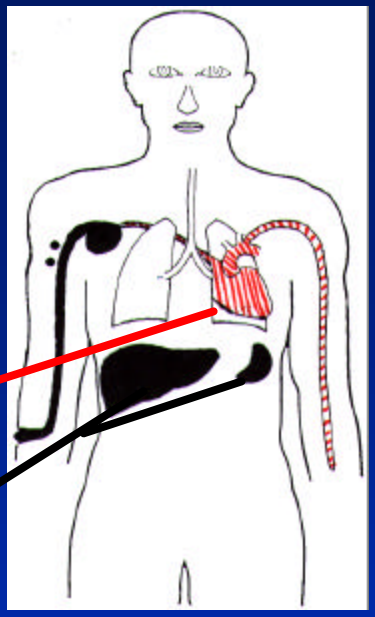


Stage 2

Yfe transport system important in later stages of disease

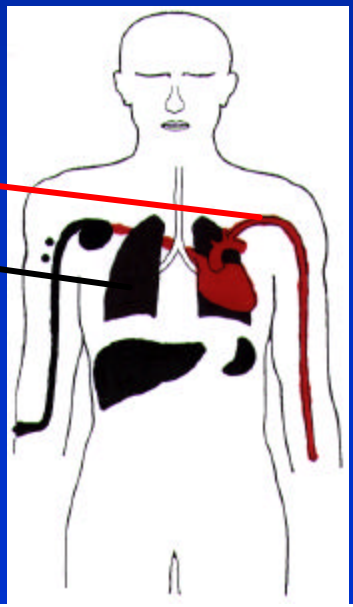
4. Hematogenous spread
5. Growth in liver and spleen



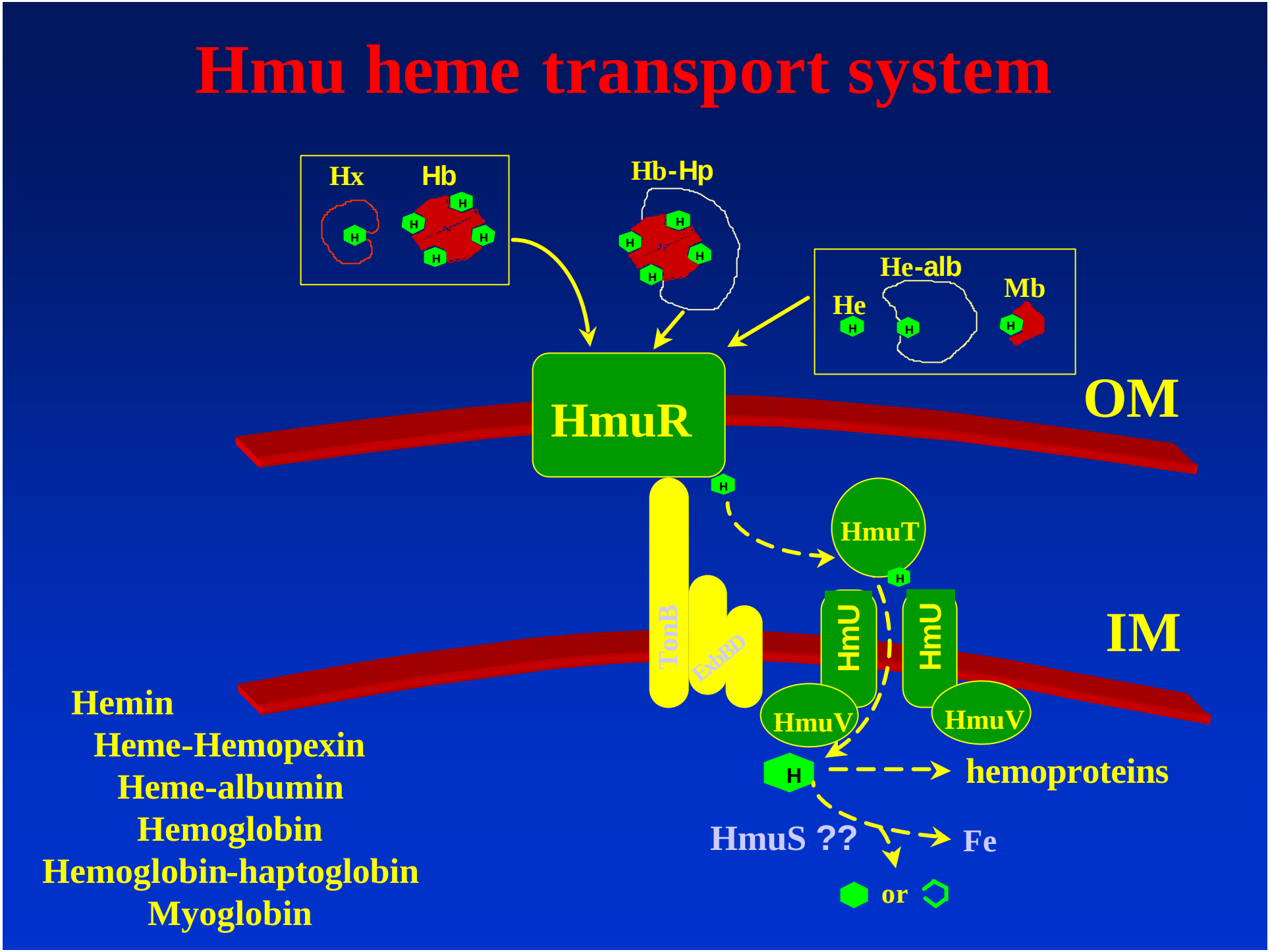
Ybt iron transport system and Pla protease essential in the early stages of bubonic plague

Stage 3

6. Septicemia
7. Lung infection (Occasionally)
8. Death



Hmu heme transport system

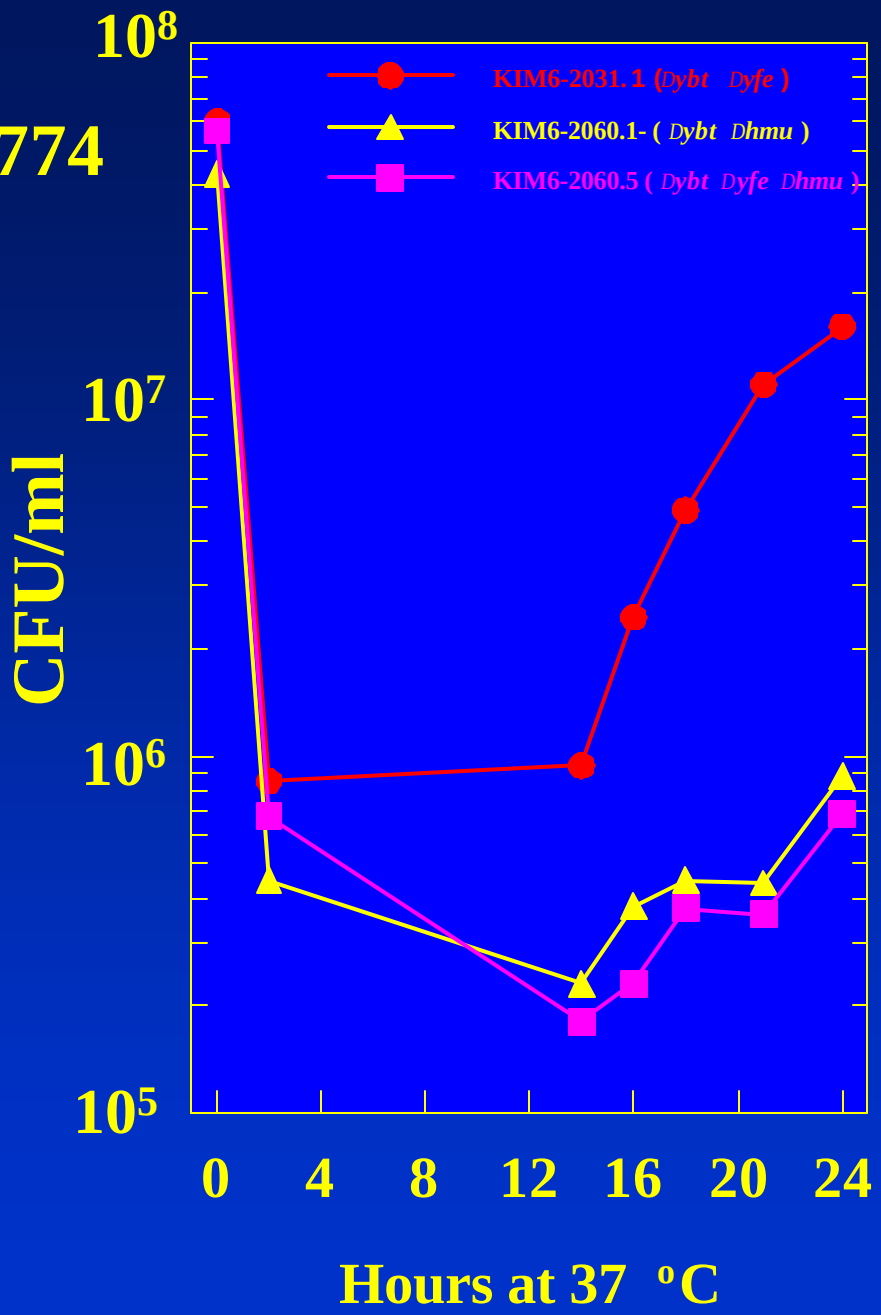


Hmu System

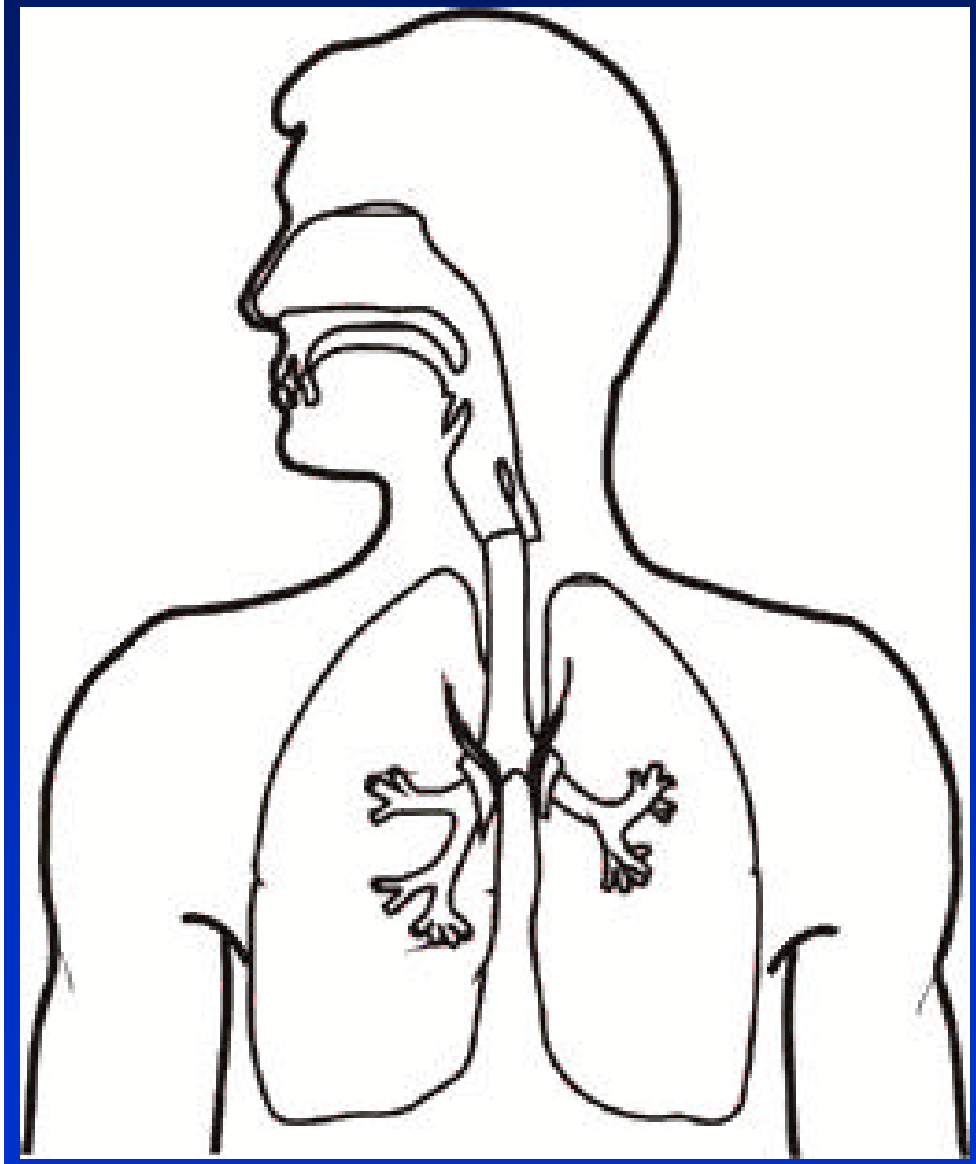
- Required for growth in J774 cells
- Not required for full virulent by S.C. route

Yfe and Feo Systems

- Yfe$^-$ Feo$^-$ mutant – reduced growth similar to Hmu$^-$ mutant
- Single mutants grow as well as parental strain



Primary Pneumonic Plague



Symptoms develop 1-3 days after exposure

- **Bronchopneumonia**
- **Becomes lobar and multilobar**
- **Gastrointestinal symptoms nausea, vomiting, abdominal pain, diarrhea**
- **~100% untreated fatality rate**
- **Treatment delayed >24 h after onset of symptoms often fatal**

Role of presumed virulence determinants in pneumonic plague

TESTED

- **Ybt (iron transport) – ~42-fold virulence loss in mice**
  Altered disease pathology and time to death
- **Pla – Plasminogen activator – 730,00-fold viruelnce loss**
  Ybt^- Pla^- double mutant completely avirulent
- **F1 capsule – No change in LD_{50}**
  Increase in time-to-death

NOT TESTED

- **Yfe Fe and Mn transport system (and Feo)**
- **Lcr (Yops and LcrV)**
- **Psa (Fibrils)**
- **Hmu heme transport system**

Potential New Subunit Vaccine Candidates

- **Pla protease**
  - + 37ºC expression; roles in adherence/invasion and spread
  - - Pla antigen was not protective (USAMRIID, unpublished)
- **Psn – OM receptor for Ybt siderophore**
  - + Essential in early stages; highly expressed
  - - Nonessential in later stages of disease
- **OM components of Yfe (and Feo?) transporter(s)**
  - + Important in later stages; highly expressed; important for intracellular growth
  - - Surface-exposed components not identified
- **Psa fibrillar subunit**
  - + 37ºC expression under acidic conditions
  - - Virulence role not defined
    Contradictory evidence on importance
    Not highly immunogenic

Potential New Subunit Vaccine Candidates

- **HmuR – OM receptor for heme/hemoproteins**
  - + **Highly expressed; essential for heme/hemoprotein use; important for intracellular growth**
  - - **No role in virulence in bubonic plague mouse model**
- **Other putative surface-exposed proteins**
  - + **Adhesins/pili; OM receptors; secreted proteins**
    Flashner *et* al. 2004. Infect. Immun. 72:908-915
    Signature-tagged mutagenesis
    Motin *et al*. 2004. J. Bacteriol. 186:6298-6305
    Identified Orfs expressed at 37°C
  - - **Not shown to be expressed or important in vivo**
- **Cell envelope carbohydrates**
  - + **F1 carbohydrate component; 37°C LOS**
  - - **F1 problems; 28°C LOS alone not protective in bubonic model**

BLACK DEATH EUROPEAN TOUR

DEC 1347	CONSTANTINOPLE
DEC 1347	MARSEILLES
JUNE 1348	NAPLES
JUNE 1348	ROME
DEC 1348	BRISTOL
JUNE 1349	LONDON
JUNE 1349	OXFORD
DEC 1349	DUBLIN
DEC 1349	YORK
JUNE 1350	MAGDEBURG
JUNE 1350	ROSTOCK HAMBURG
DEC 1350	WISBY
DEC 1350	DANZIG